09/977,716

WEST

Freeform Search

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Term: l7 and stain$3

Display: 10 **Documents in Display Format:** - **Starting with Number** 1

Generate: ○ Hit List ◉ Hit Count ○ Side by Side ○ Image

Search | Clear | Help | Logout | Interrupt

Main Menu | Show S Numbers | Edit S Numbers | Preferences | Cases

Search History

DATE: Thursday, August 07, 2003 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L10	l7 and stain$3	25	L10
L9	RNA near5 (fluorescen$2 or ethidium) near5 direct$2 near5 stain$3	1	L9
L8	l6 and immuno RNA	0	L8
L7	L6 and RNA amplification	39	L7
L6	RNA near5 (fluorescen$2 or ethidium)	1209	L6
L5	L4 and direct$3	0	L5
L4	L3 and (stain$3 near5 direct$3)	0	L4
L3	l1 and fluorescen$2	2	L3
L2	L1 and fluorescent and stain$3	0	L2
L1	immuno RNA	5	L1

END OF SEARCH HISTORY

Generate Collection | Print

Search Results - Record(s) 11 through 20 of 25 returned.

☑ 11. 5985572 . 23 Feb 98; 16 Nov 99. Quaternary amine surfactant and methods of using same in isolation of RNA. Macfarlane; Donald E.. 435/6; 536/23.1. C12Q001/68.

☐ 12. 5958698 . 17 Aug 98; 28 Sep 99. Method for amplification and expression of nucleic acids in solid media and its application for nucleic acid cloning and diagnostics. Chetverin; Alexander Borisovich, et al. 435/6; 435/91.2. C12Q001/68 C12P019/34.

☐ 13. 5939265 . 05 Nov 97; 17 Aug 99. Reagents and methods useful for detecting diseases of the lung. Cohen; Maurice, et al. 435/6; 435/320.1 435/325 536/23.1 536/23.5. C12Q001/68 C12N015/00 C12N015/63 C12N015/85.

☐ 14. 5843640 . 30 Aug 95; 01 Dec 98. Method of simultaneously detecting amplified nucleic acid sequences and cellular antigens in cells. Patterson; Bruce, et al. 435/5; 435/6 435/7.1 435/91.2. C12Q001/70 C12Q001/68.

☐ 15. 5840478 . 18 May 94; 24 Nov 98. Method of detecting amplified nucleic sequences in cells by flow cytometry. Patterson; Bruce, et al. 435/5; 435/6 435/91.2. C12Q001/70 C12Q001/68 C12P019/34.

☐ 16. 5763167 . 21 Mar 94; 09 Jun 98. Applications of fluorescent N-nucleosides and fluorescent structural analogs of N-nucleosides. Conrad; Michael J.. 435/6; 536/24.3 536/26.13 536/28.4. C12Q001/68.

☐ 17. 5728822 . 07 Sep 95; 17 Mar 98. Quaternary amine surfactants and methods of using same in isolation of RNA. Macfarlane; Donald E.. 536/25.41; 564/281. C07H021/00.

☐ 18. 5728525 . 02 Jun 95; 17 Mar 98. Fluorescent universal nucleic acid end label. Conrad; Michael J.. 435/6; 435/91.1 536/23.1 536/24.3 536/24.33 536/25.3. C12Q001/68 C07H021/04.

☐ 19. 5652099 . 18 Aug 94; 29 Jul 97. Probes comprising fluorescent nucleosides and uses thereof. Conrad; Michael J.. 435/6; 536/24.3 536/24.31 536/24.32 536/24.33 536/26.23 536/26.6 536/26.7 536/27.13 536/27.6 536/28.2 536/28.5. C12Q001/68 C07H021/04.

☑ 20. 5631129 . 16 May 95; 20 May 97. Target nucleic acid amplification/detection systems and methods for the use thereof. Chu; Barbara C., et al. 435/5; 435/6 435/810 435/91.1 435/91.2 435/91.21 435/91.3 435/91.5 435/91.51 436/501 536/22.1 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33 536/25.3 536/25.4. C12Q001/68 C07H021/02 C07H021/04.

Generate Collection | Print

☐ Generate Collection

L10: Entry 20 of 25 File: USPT May 20, 1997

DOCUMENT-IDENTIFIER: US 5631129 A
TITLE: Target nucleic acid amplification/detection systems and methods for the use thereof

Brief Summary Text (13):
It is an object of the present invention to provide compositions and methods for detection of RNA target segments using a process that, in assuring the presence of the target sequence in the amplified product, avoids or at least substantially reduces the presence of low signal-to-noise ratios and false positives. It is a further object of the present invention to provide target RNA amplification compositions which utilize portions of autocatalytically replicatable RNAs and which result in the production of recombinant replicatable RNAs, such that autocatalytic replication of the recombinant RNAs results in amplification of a target nucleic acid of interest (i.e., a segment of the target RNA) which is foreign to the replicatable RNA.

Detailed Description Text (36):
RNA resulting from the replication process can be made fluorescent by employing a T4 RNA ligase catalyzed reaction to append nucleotides modified to be fluorescent to the 3'-end of replicative RNA. See Cosstick et al., Nucl. Acids Res. 12, 1791 (1984). The fluorescence of the resulting RNA can be employed to detect the RNA by any of several standard techniques.

Detailed Description Text (37):
Among still other methods that can be used to detect replicated RNA are those wherein a reporter substance, that binds specifically with nucleic acid, is added to the system in which the replication has taken place, or to the medium, such as a positively charged support such as ECTEOLA paper, on which replicated RNA has been isolated, and signal from the reporter substance measured. Such substances include: chromogenic dyes, such as "stains all" (Dahlberg et al., J. Mol. Biol. 41, 139 (1969); methylene blue (Dingman et al., Biochemistry 7, 659 (1968), and silver stain (Sammons et al., Electrophoresis 2, 135 (1981); Igloi, Anal. Biochem. 134, 184 (1983)); fluorogenic compounds that bind to RNA--for example, ethidium bromide (Sharp et al., Biochemistry 12, 3055 (1973); Bailey et al., Anal. Biochem. 70, 75 (1976); and fluorogenic compounds that bind specifically to RNAs that are templates for replication by Q.beta. replicase--for example, a phycobiliprotein (Oi et al., J. Cell Biol. 93, 981 (1982); Stryer et al., U.S. Pat. No. 4,520,110) conjugated to the viral subunit of Q.beta. replicase.

Detailed Description Text (49):
The amount of RNA is determined by its intrinsic UV absorbance (e.g. as by the contact photoprinting method of Kutateladze et al., Anal. Biochem. 100, 129 (1979)). Alternatively, the RNA is visualized on ETEOLA paper. Aliquots (of equal volume) of replication reaction are transferred with 13, 48 or 96-fingered aliquotter to sheets of diethylaminoethyl cellulose paper. The sheets are then washed at room temperature in a solution of 200 mM NaCl, 300 mM ammonium acetate pH 6 to remove ribonucleoside triphosphates not incorporated into RNA. The sheets are then stained with 0.3 .mu.g/ml of ethidium bromide. (Sharp et al., Biochemistry 12, 3055 (1973); Bailey et al., Anal. Biochem 70, 75 (1976).

Detailed Description Text (50):
Finally the fluorescence from individual blots is measured by any of several known techniques. Fluorescence intensity from a stained blot above that from control blots indicates the presence of target. Other staining materials can be employed in place of ethidium bromide. These include methylene blue (Dingman and Peacock, Biochemistry 7, 659 (1968)), silver stain (Sammons, et al., Electrophoresis 2, 135 (1981)) or phycobiliprotein Q.beta. replicase conjugate (Oi et al., J. Cell Biol. 93, 981

☐ Generate Collection

L10: Entry 11 of 25 File: USPT Nov 16, 1999

DOCUMENT-IDENTIFIER: US 5985572 A
**** See image for Certificate of Correction ****
TITLE: Quaternary amine surfactant and methods of using same in isolation of RNA

Detailed Description Text (33):
Examination of the gel under ultraviolet light after staining with ethidium bromide showed the presence of rRNA and other RNA in the lanes loaded with the 100 microliter samples of blood. The lanes loaded with 400 microliters blood revealed RNA that was partially degraded. There was no difference between the lanes containing samples incubated with the surfactant for 0, 15 minutes, 30 minutes, or 1 hour.

Detailed Description Text (51):
After cooling to -20.degree. C. for 30 minutes, the precipitated RNA was harvested by centrifugation (16,000 g, 5 minutes), washed with ethanol, and redissolved for analysis by agarose gel electrophoresis. This revealed the characteristic ethidium bromide stainable bands of cellular RNA. This experiment shows that RNA can be extracted from the surfactant nucleic acid pellet by high salt concentrations. Guanidinium isothiocyanate is known to inhibit RNase, which action may facilitate the recovery of RNA.

Detailed Description Text (61):
An experiment similar to Example 11 was performed, except that the guanidinium method of Example 10 was used to isolate the RNA An amplified product of the appropriate size was seen when RNA from blood samples contained 30 or more K562 cells. This experiment shows that extracting the surfactant nucleotide complex with guanidinium isothiocyanate as described yields RNA which is suitable for amplification without further purification. As described, this method would appear to be capable of detecting less than one leukemic cell per microliter of blood of patients with chronic myelogenous leukemia having the Philadelphia chromosome, and this illustrates the great sensitivity of this method.

Generate Collection

L10: Entry 3 of 25 File: USPT Apr 9, 2002

DOCUMENT-IDENTIFIER: US 6369207 B1
TITLE: Nucleic acid amplification with DNA-dependent RNA polymerase activity of RNA replicases

Detailed Description Text (13):
Any of numerous methods can be employed to assay for reporter RNA (or its complement). In situations where the mass of reporter RNA and its complement, if made, will be substantial fraction of the mass of nucleic acid present after the amplification, a nucleic acid-staining dye can simply be added to an aliquot of sample in which the amplification was carried out and the aliquot can be visualized to see whether staining has occurred. Staining will occur and be observed only if target nucleic acid was present to lead to production of reporter RNA. Situations in which this simple staining technique can be applied include those where, after amplification, the stained reporter RNA and its complement are visible in an aliquot of sample and the mass of such stained reporter RNA and complement exceeds by a factor of at least about two the mass of other nucleic acid present in the sample after the amplification.

Detailed Description Text (14):
In situations where the amount of reporter RNA and its complement formed in the amplification process is too low to allow simple staining to be used to detect whether target nucleic acid was present in a sample, the nucleic acid of an aliquot of a sample, after the amplification process is carried out with the sample, can be separated by size, e.g., electrophoretically, and then stained. Production in the amplification process of nucleic acid of the size of reporter RNA and its complement, as detected by observing stained nucleic acid of that size in the size-separated nucleic acid, indicates that target nucleic acid was present in the sample of nucleic acid being analyzed.

Detailed Description Text (20):
These kits of the invention may be test kits for detecting the presence of a target nucleic acid analyte, comprising a pre-selected target segment, in a test sample thought to contain said analyte. Such test kits comprise additionally reagents for rendering detectable reporter RNA or complement thereof produced in the amplification carried out with the components of the kit on an aliquot of the test sample if said sample comprises said analyte. In the test kits, such reagents will be held in detection-reagent-holding containers that are packaged together with the replicase-holding, probe-holding and any enzyme-holding containers. Such reagents, which might be included in a test kit, include, for example, a solution of a dye to stain nucleic acid, a solution of a nucleic acid probe that is labeled for detection and that is capable of hybridizing to reporter RNA or complement thereof, or a solution of a beetle luciferase.

Detailed Description Text (135):
The detection of amplified products can be performed by methods and materials familiar to those skilled in the art. Such detection methods include reactions of RNA with dyes and detection of the dye-RNA complexes. Especially in situations where the RNA amplification product is present in a significant background of other nucleic acids, which would also form complexes with a dye, detection of amplification product by formation of dye-RNA complexes can be accompanied by separation (as by electrophoresis, chromatography or the like) according to size of nucleic acid of a sample thereof in which an amplification reaction has been carried out in order to detect the product(s) of the amplification reaction, which will have characteristic size(s). Confirmation that nucleic acid of the expected size found in a sample using dye-staining after an amplification reaction according to the invention is RNA from the amplification reaction can be obtained by using a sequence-specific detection method, such as a nucleic acid probe hybridization method, as described below. The

dyes include chromogenic dyes such as "stains all" (Dahlberg, et al. (1969), J. Mol. Biol., Vol 41, pp. 139-147), methylene blue (Dingman and Peacock (1968), Biochemistry, Vol. 7, pp. 659-668) and silver stain (Sammons, et al. (1981), Electrophoresis, Vol. 2, pp. 135-141; Igloi (1983), Anal. Biochem., Vol. 134, pp. 184-188) and fluorogenic compounds that bind to RNA, including ethidium bromide (Sharp, et al. (1973), Biochemistry, Vol. 12, pp. 3055-3063; Bailey and Davidson (1976), Anal. Biochem., Vol. 70, pp. 75-85), acridine orange, propidium iodide and ethidium heterodimer.

Detailed Description Text (141):
The separation of RNA produced in amplification by autocatalytic replication and containing either normal or modified nucleotides or bound with dyes is generally conducted by methods and means known to the art. For example, amplified materials can be bound to filters or particles and unbound modified nucleotides or dyes can be separated and removed by suitable washing conditions. The binding process can be non-specific, e.g., binding all nucleic acids, but not unincorporated materials; or specific, binding only nucleic acids comprising particular sequences. or other properties. Specific binding can be directed by substances that are bound to any of various support materials (e.g., surfaces of wells on microtiter plates, latex or agarose beads (including magnetic beads), chromatographic resins, as understood in the art) and that are capable of complexing specifically with certain nucleic acids. For example, when the nucleic acid to be specifically bound is RNA amplification product resulting from amplification in accordance with the invention, such specific-binding substances include antibodies to specific classes of nucleic acids, e.g., double-stranded RNA, nucleic acids comprising a segment with a specific sequence complementary to a sequence in amplified product; or avidin or streptavidin to complex with biotin in the RNA produced in the amplification process as described previously.

Detailed Description Text (205):
The product of the amplification reaction described in Example 1 was also analyzed by electrophoresis on an 8% polyacrylamide, 7M urea denaturing gel according to the following procedure. A 30 ml gel (0.4 mm thick) was prepared by mixing 76 g/l acrylamide, 8 g/l bis-acrylamide, 440 g/l urea, 500 .mu.l/l TEMED in 1.times.TBE (1.times.TBE: 89 mM Tris base, 89 mM boric acid, 2 mM EDTA). To 30 ml of this solution was added 500 .mu.l of a fresh solution of 10% ammonium persulfate. After gel polymerization, 5 .mu.l samples of the nv(+)DNA amplification reaction mixture (which included 25 attomoles of nv(+) DNA) were treated, prior to loading on the gel, by being heated to 95.degree. C. for 2 minutes in 20 .mu.l blue juice (blue juice: 600 mg/ml urea, 1 mM EDTA, 5% glycerol, 0.05% bromophenol blue, 0.05% xylene cyanol). The gel was prerun for 30 minutes at 300 volts and, after loading of samples, was run at 400 volts for 1 hour. The gel was then stained for 20 minutes in a solution of 0.5 .mu.g/ml ethidium bromide and nucleic acids were visualized by fluorescence, caused by exposure of the gel to ultraviolet light at 302 nm.

Detailed Description Text (207):
The procedure for determining that the amplified material included both strands of RNA was as follows. Two stained gels were prepared as described above. Each was soaked for 20 minutes in 0.1 .times.TBE containing 0.5 .mu.g/ml ethidium bromide and the nucleic acid electrophoretically transferred to a Hybond membrane filter (Amersham, Cat. No. RPN. 203N, Arlington Heights, Ill., USA) for 10 minutes at 50 volts in 0.1.times.TBE. The filter was washed for 30 minutes at 65.degree. C. in 0.1.times.SSC (1.times.SSC: 150 mM sodium chloride, 15 mM sodium citrate), 0.5% SDS. The filter was then prehybridized for 3 hours at 65.degree. C. in 5.times.SSC, 40 mM NaPO.sub.4, 5.times.Denhardt's Solution (1.times.Denhardt's Solution: 200 .mu.g/ml each of ficoll, polyvinylpyrrolidone, and bovine serum albumin (BSA)), 0.1 mg/ml sheared and denatured herring sperm DNA, and 10% dextran sulfate. .sup.32 PO.sub.4 -kinased oligonucleotide PM618 (a 66-base DNA probe with the sequence of SEQ ID NO: 17, which is the same as that of the 66 bases at the 5'-end of nv(+)DNA) and .sup.32 PO.sub.4 -kiased oligonucleotide PM624 (a 66-base DNA probe with the sequence of SEQ ID NO: 18, which is complementary to the sequence of the 66 bases at the 3'-end of nv(+)DNA), respectively, were heated to 95.degree. C. for 5 minutes and added for hybridization to the separate filters that had been prepared, washed and prehybridized as described, supra, (except that the filter used with PM618 had been previously used with another probe buT this probe had been stripped by exposure of the filter to three sequential 1 minute treatments at 100.degree. C. with 0.1.times.SSC, 0.1% SDS). The mixtures were hybridized overnight at 65.degree. C. Then, the filters were rinsed briefly with

2.times.SSC, 0.1% SDS, again with the same solution at room temperature for 15 minutes and again for 30 minutes. The filters were rinsed two more times for 30 minutes each at 65.degree. C. in 0.1.times.SSC, 0.1% SDS and were exposed to Kodak XAR-5 film at -80.degree. C. using two DuPont Cronex Hi Plus intensifier screens.

Detailed Description Text (208):
The results indicated strong hybridization of both the PM618 and PM624 to the products of the amplification reaction seen by ethidium stain described above. This indicates that both strands of nvRNA are made, fulfilling the final requirement of autocatalytic replication.

Detailed Description Text (216):
The reaction products were analyzed following electrophoresis through an 8% polyacrylamide 7M urea denaturing gel according to the following procedure. A 40 ml gel (1.5 mm thick) was prepared by mixing 76 g/l acrylamide, 4 g/l bis-acrylamide, 500 g/l urea in 1.times.TBE. To 50 ml of this solution were added 25 .mu.l of TEMED and 250 .mu.l of a fresh solution of 10% ammonium persulfate. After gel polymerization, 5 .mu.l samples of amplification reaction mixtures were prepared by heating to 95.degree. C. for 1 minute in 25 .mu.l blue juice (Example 2). The gel was prerun for 30 minutes at 30 mA and, after loading samples, was run at 30 mA for 1.5 hours. The gel was stained and visualized as described in Example 2. When the target, PM2123, was present in the reaction, two major bands, of approximately 118 and 110 bases in length, and at least 7 weaker bands, with lengths from about 80 to several hundred bases, were observed. These data indicate that the amplification of reporter molecules was dependent on the presence of target molecules in the sample.

Detailed Description Text (217):
Confirmation of target-specific amplification was demonstrated by hybridization of the electrophoretically separated material with probe PM407 according to the following procedure, For the sequence of PM407, see SEQ ID NO: 19 in the Sequence Listing. Nucleic acid from the stained gel was electrophoretically transferred to a Hybond membrane filter for 20 minutes at 45 volts in 0.1.times.TBE. The resulting filter was prehybridized for 1 hour at 65.degree. C. as described in Example 2. .sup.32 PO.sub.4 -kinased oligonucleotide PM407 was added and the mixture was hybridized for 4 hours at 60.degree. C. The filter was rinsed for one minute at room temperature in 2.times.SSC, 0.1% SDS and 5 times for 15 minutes each at 60.degree. C. in 2.times.SSC, 0.1% SDS. The resulting filter was exposed to Kodak XAR-5 film at -80.degree. C. using two DuPont Cronex Hi-Plus YE intensifier screens. Hybridization was observed only with the 118-base band.

Detailed Description Text (233):
Referring now to Table 2 below it will be seen that after hybridization with M13mp19 phage DNA, the hybrid DNA peak fraction contained 1230 cpm and produced 1485 fluorescence units of RNA after Q.beta. amplification. The specificity of the hybridization and detection steps were confirmed by the use of a nonhomologous mock target DNA (.phi.X174 phage DNA). The peak that would have contained hybrid that was eluted from the Bio Gel A-5 column after hybridization with .phi.X174 DNA contained only a background level of radioactively-labeled probe and no detectable RNA was produced by Q.beta. amplification.

Detailed Description Text (240):
The products of the amplification were analyzed on an 8% denaturing polyacrylamide gel as described in Example 2. The separated products were electrophoretically transferred to a Hybond nylon filter (Amershain, Cat. No. RPN. 203N) for 20 minutes at 40 volts in 0.1.times.TBE. The filter was visualized under ultraviolet light at 302 nm to confirm transfer of the stained products. RNA products on the filter were cross-linked to the filter by exposing the filter to 1200 .mu.J of ultraviolet light at 254 nm using a Stratalinker 1800 (Stratagene, Cat. No. 400071, La Jolla, Calif., USA). The filter was then prehybridized for one hour at 65.degree. C. in 20 ml of hybridization solution B (5.times.SSC, 10% dextran sulfate, 100 .mu.g/ml denatured herring sperm DNA, 40 mM NaPO.sub.4, and 5.times.Denhardt's Solution). The probe for this hybridization was PM407 (see Table 1), with the sequence of SEQ ID NO: 19. Oligonucleotide PM407 corresponds to the Salmonella sequence present in oligonucleotide PM1059. Hybridization with this probe indicates presence of amplified ligated products because PM1059, alone, is not ampliflable. Probe PM407 was kinase labelled with .sup.32

PO.sub.4 for 1 hour at 37.degree. C. in a 10 .mu.l volume. After heat killing the kinase at 90.degree. C. for three minutes, the entire labelling reaction mixture was added to the hybridization solution and filter. The hybridization proceeded for four hours at 60.degree. C. The filter was rinsed briefly with wash solution (2.times.SSC, 0.1% SDS) at room temperature followed by 5 15-minute washes with wash solution at 60.degree. C. The filter was then exposed to Kodak XAR-5 film at -80.degree. C. for 16 hours using two DuPont Cronex Lightning Plus intensifier screens.